CURRENT LITERATURE

NOTES FOR STUDENTS

Estimation of plant carbohydrates.—In a series of papers from the Rothamstead Experimental Station, DAVIS, DAISH, and SAWYER have reported the results of a critical study of existing methods for the estimation of carbohydrates in plant extracts, and have suggested a number of improvements and modifications designed to secure greater accuracy. In the initial paper of the series, on the estimation of maltose in solution with other sugars, DAVIS and DAISH[1] point out several sources of possible error in the gravimetric method of BROWN, MORRIS, and MILLAR. All samples of asbestos examined, even after previous washing in acid and ignition, contained an easily decomposed silicate, which was rapidly dissolved by the hot alkaline Fehling's solution, thus giving weights which were uniformly too low. Digesting the asbestos for 30 minutes with boiling 20 per cent NaOH and subsequent washing with water removes all material soluble in Fehling's solution, and the authors recommend such digestion as a routine procedure. Since the precipitate of cuprous oxide obtained from plant extracts or from solutions previously subjected to fermentation by yeasts invariably contains copper salts of amino acids and adsorbed colloidal organic matter, the employment of the official method of weighing cuprous oxide as such after drying at 100° introduces an error. This is partially corrected by placing the crucible, after previous washing with alcohol and ether and drying at 100° in a larger crucible and heating for 30 minutes, without the use of a blowpipe, in a powerful flame, subsequently weighing as cupric oxide.

Of the more generally employed volumetric methods subjected to test, the Ling-Rendle method, employing an acid solution of ferrous ammonium sulphate and ammonium thiocyanate as indicator for Fehling's solution, is accurate to at least 0.3 per cent, and is also much more rapid than the Bertrand permanganate method. The latter was found to have an error of 1 per cent with maltose, 1.5 per cent with dextrose, while the results for cane sugar were 3.5 per cent low, as the 2 per cent HCl used for inversion caused considerable decomposition of levulose. In estimating cane sugar in solutions containing maltose, it is impossible to use HCl at 70° for inversion, since maltose is also hydrolyzed, while pentoses undergo decomposition; nor can 2 per cent citric acid be employed, since the use of basic lead acetate to precipitate tannins, amino acids, etc., and the subsequent precipitation of lead with Na_2CO_3,

[1] DAVIS, WILLIAM A., and DAISH, ARTHUR JOHN, A study of the methods of estimation of carbohydrates, especially in plant extracts. I. A new method for the estimation of maltose in presence of other sugars. Jour. Agric. Sci. 5:437–468. 1913.

leaves in solution a quantity of sodium acetate sufficient to practically inhibit inversion by 2 per cent acid. The authors find that 10 minutes' boiling with 10 per cent citric acid completely hydrolyzes cane sugar without affecting maltose or decomposing pentoses, and recommend this method. Inversion by invertase also gave quantitative results which were too low, apparently, because maltose is carried down in the precipitation with alumina cream. It was impossible to estimate maltose in plant extracts by hydrolysis for 3 hours with dilute boiling HCl or H_2SO_4, as recommended by BROWN and MORRIS, since there was destruction of at least 30 per cent of the levulose present, with measurable amounts of dextrose, by such treatment with any concentration of acid sufficient to effect complete hydrolysis of the maltose present. Hydrolysis with 2.44 per cent HCl at 70° gave no better results; in a 1 per cent solution only 94 per cent of the maltose had been converted after 24 hours' boiling and there had been material destruction of the levulose present. The authors therefore adopted fermentation of the maltose-containing solution with pure cultures of maltose-free yeast as the only satisfactory procedure. The solution is freed of tannins, amino acids, etc., with basic lead acetate, is then made lead-free by adding solid Na_2CO_3, filtering, acidifying, treating with H_2S, and finally making slightly acid to litmus with dilute Na_2CO_3. Three yeasts, *Saccharomyces exiguus*, *S. anomalus*, and *S. marxianus*, were used, the fermentation being continued at 25° for 31 days. All gave good results, but *S. exiguus* is best for general use, since it is least sensitive to acid and its less bulky growth causes less contamination of the cuprous oxide precipitate with salts of amino acids. Checks fermented with ordinary distillery yeasts permit the making of a correction for pentoses remaining after the other sugars have been destroyed. Pentoses were determined by distillation of an aliquot of the solution with HCl at 70° and weighing the furfural as phloroglucide.

The authors' assertion that maltose is hydrolyzed by HCl at 70° has been questioned by KLUYVER,[2] and DAVIS has consequently presented further evidence[3] by reporting the results of a series of experiments with a 1 per cent solution of maltose, carried out under exact Herzfeld conditions, which show a rather uniform loss by hydrolysis of about 2 per cent of the maltose present.

The authors present a scheme for the analysis of plant extracts which may be summarized as follows. The extract is evaporated to small volume in vacuo and made up to 500 cc. Duplicate 20 cc. portions are evaporated to dryness and the drying completed in vacuo for dry matter determinations. The remainder of the solution is treated with basic lead acetate, filtered, and made up to 2000 cc. A portion of this is freed of lead, made up to convenient

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[2] KLUYVER, A. J., Biochemische Suikerbepalingen. pp. 223. Boekhandlung E. J. Brill, Leiden. 1914.

[3] DAVIS, WILLIAM A., The hydrolysis of maltose by hydrochloric acid under the Herzfeld conditions of inversion. A reply to A. J. KLUYVER. Jour. Agric. Sci. 6:413–416. 1914.

volume, and divided into two portions. Upon one of these a determination of direct reduction, representing total dextrose, levulose, maltose, and pentoses, is made; the other is employed for determination of cane sugar by inversion with 10 per cent citric acid and with invertase. The remainder of the 2000 cc. of solution is freed of lead and divided into portions. Upon one of these maltose is determined by fermentation with *S. exiguus* or other maltase-free yeast, checked by fermentation with ordinary yeast; the remaining portion is distilled with HCl for the determination of pentoses.

The second paper of the series deals with the methods of estimating starch in plant material.[4] The modified Sachsse method, in which starch is hydrolyzed by boiling HCl, is said to be valueless for two reasons: such plant materials as leaves and seeds contain pentosans and other compounds which are broken down, yielding reducing sugars which are computed as dextrose, while the prolonged boiling with acid destroys some of the dextrose present. O'SULLIVAN'S method of estimating starch by converting it into a mixture of dextrin and maltose by the use of ordinary diastase is also shown to give rise to low results; plant material freed of sugar by alcohol extraction still contains tannins, amino acids, and other compounds which necessitate precipitation with basic lead acetate, and a considerable quantity of the dextrin present (15–20 per cent under the conditions of the experiments) is carried down by the lead precipitate and thus lost to the analysis. The authors show that this loss of dextrin is avoided by the use of taka-diastase. When taka-diastase is allowed to act for 6 hours at 38° upon previously gelatinized starch, the whole of the starch is converted into a mixture of maltose and dextrose, continued action resulting in a steady increase in the amount of dextrose, until final equilibrium is attained. The authors therefore adopt the following method. Material for analysis is prepared by dropping the freshly collected leaves or other parts into boiling 95 per cent alcohol to which 1 per cent of concentrated ammonia has been added; immediate destruction of all enzymes is thus assured. Sugars are removed by 18–24 hours' continuous extraction in a special apparatus of the Soxhlet type; the material is freed of alcohol by pressing in a Buchner press and drying 18 hours in a steam oven. It is then ground and bottled for analysis. Samples taken for analysis are dried in vacuo before beginning actual work upon them. As leaf materials usually contain considerable quantities of gum, amylans, and other non-starch constituents which yield reducing sugars, it is necessary to remove these by extraction with a large volume of water for 24 hours at 38°, followed by thorough washing. The material is now boiled with water 30 minutes to gelatinize the starch, cooled to 38°, taka-diastase added (0.1 gm. for 10 gms. vacuum-dried material), and the mixture kept for 24 hours at 38° after the addition of a little toluene. The diastase is then

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[4] DAVIS, WILLIAM A., and DAISH, ARTHUR JOHN, Methods of estimating carbohydrates. II. The estimation of starch in plant material. The use of taka-diastase. Jour. Agric. Sci. **6**:152–168. 1914.

destroyed by boiling, the residuum is filtered, washed, the solution made to volume, precipitated with lead, using care to avoid an excess, and portions are taken for polarization and for reduction. Values for the maltose-dextrose mixture are then calculated from the tables of BROWN, MORRIS, and MILLAR.

DAISH[5] has determined the cupric reducing power of xylose and arabinose under the standard conditions prescribed by BROWN, MORRIS, and MILLAR, as all previously published values were determined under somewhat different conditions. He presents tables of the reducing power of each of these sugars for quantities between 10 and 200 mgm. Two curves obtained by plotting the reducing power, expressed as CuO, against the weight of sugar employed are given; from these curves it is possible to determine the weight of sugar corresponding to any given weight of CuO by the employment of a divisor number. The reducing powers of xylose and arabinose are almost identical and differ very little from that of dextrose; thus for 100 mgm. of sugar the divisor number for dextrose is 2.358, for arabinose 2.536, and for xylose 2.490.

DAVIS and SAWYER[6] have presented evidence that free pentoses are quite generally present in the alcoholic extracts of plant material. This evidence they summarize; there are present substances which are soluble in 80 per cent alcohol, which are not precipitable by basic lead acetate, which are not fermentable by ordinary yeasts, and which give the solution reducing power after all fermentable sugars have been destroyed by yeast. This reducing power, if calculated as that of a mixture of xylose and arabinose, agrees almost exactly with the pentose value of the phloroglucide obtained by a KRÖBER-TOLLENS distillation of the solution after previous precipitation with basic lead acetate. These facts can only be explained upon the assumption that the furfural obtained in distillation is derived from free pentoses, not from pentosans, gums, or other sugars.

Various plants, as marigold, turnip, carrot, potato, *Helianthus*, and *Tropaeolum*, showed the presence in the leaves of pentoses in amounts ranging from 0.3 to 1.0 per cent of the total vacuum-dried material, when determinations were made by the KRÖBER-TOLLENS method upon material prepared according to the authors' method. The presence of other sugars, as cane sugar, in the solution to be distilled gives results which are considerably above the true pentose content. Consequently it is necessary, when very accurate determinations of pentoses are desired, to remove the other sugars by fermenting with *Saccharomyces cerevisiae* and to make the determination by distillation of the fermented solution.

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[5] DAISH, ARTHUR JOHN, Methods of estimation of carbohydrates. III. The cupric reducing power of the pentoses xylose and arabinose. Jour. Agric. Sci. **6**: 255–262. 1914.

[6] DAVIS, WILLIAM A., and SAWYER, GEORGE CONWORTH, The estimation of carbohydrates. IV. The presence of free pentoses in plant extracts and the influence of other sugars on their estimation. Jour. Agric. Sci. **6**:406–412. 1914.

The most recent paper of the series[7] reports the results of an investigation of the generally accepted idea that an excess of basic lead acetate, when added to a solution of invert sugar, precipitates a portion or all of the levulose present as a soluble lead salt. This idea is shown to be incorrect; levulose in dilute solutions is not precipitated by basic lead acetate, even in the presence of chlorides, sulphates, or carbonates. If the acetate be added in excess and allowed to act upon the sugars for a considerable length of time, the amount of levulose present decreases progressively with increase in the time during which the lead is allowed to act. This is due to the formation from the levulose, not of a lead salt, but of a substance having a lower reducing power and much less optical activity than has levulose. It is suggested that this substance may be glutose, which was made by LOBRY DE BRUYN and VAN EKENSTEIN by heating a 20 per cent levulose solution with lead hydroxide at 70–100°, and which was described by them as having about one-half the reducing power of dextrose and as possessing only very slight optical activity. DAVIS considers that basic lead acetate acts at ordinary temperatures in the same way as does lead hydroxide, the action becoming more rapid as the temperature rises.

In order to avoid any loss of levulose when clearing a solution, the basic lead acetate must be added little by little in the cold until precipitation of the impurities is just complete, care being taken that the excess employed is not greater than 1 cc. per 100 cc. of sugar solution (best accomplished by making preliminary tests upon small portions of the filtrate). The solution should at once be filtered through a Buchner funnel, washed, and the excess of lead immediately precipitated by the use of Na_2CO_3 or Na_2SO_4. If excess of Na_2CO_3 be avoided and the solution be shaken up with a little toluene, it may be kept for months without the occurrence of any change. This treatment is very much to be preferred to the use of normal lead acetate, which fails to wholly remove optically active gums and which is a poor clarifying agent, but it is essential to accuracy that the precipitation be conducted in the cold.

The papers here reviewed were preliminary to a series on the formation and translocation of carbohydrates in plants, to be reviewed later.—JOSEPH S. CALDWELL.

Taxonomic notes.—ARTHUR,[8] in continuation of his studies of the Uredineae, has described 23 new North American species in the following genera: *Uromyces* (2), *Puccinia* (8), *Aecidium* (10), *Uredo* (3). The majority of them are from Mexico and Central America.

ASHE[9] has described a new *Vaccinium* (*V. Margarettae*) from the mountains of Georgia and South Carolina, where it occurs in association with *V. vacillans*.

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[7] DAVIS, WILLIAM A., The estimation of carbohydrates. V. The supposed precipitation of reducing sugars by basic lead acetate. Jour. Agric. Sci. **8**:7–15. 1916.

[8] ARTHUR, J. C., New species of Uredineae. X. Bull. Torr. Bot. Club **45**:141–156. 1918.

[9] ASHE, W. W., Notes on southern woody plants. Torreya **18**:71–74. 1918.